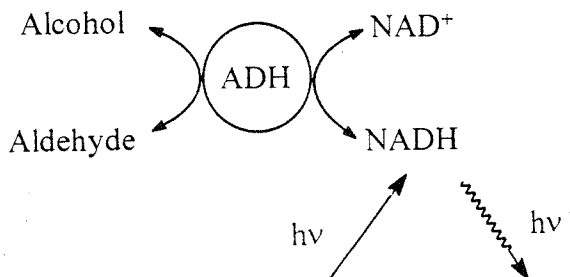


Solution and Gas Phase Sensing of Alcohols and Aldehydes: Applications of a Sol-Gel Encapsulated Enzyme System. Amy K. Williams and Joseph T. Hupp, Department of Chemistry Northwestern University, 2145 Sheridan Road, Evanston, IL 60208.

Sol-gel based encapsulation of delicate molecules and biomolecules such as enzymes and coenzymes in transparent, porous silicate matrices is now proven technology.¹ The comparatively mild room temperature processes of hydrolysis and condensation of semi-metal alkoxides produce liquid sols that can be buffered to pH's compatible with retention of enzyme activity. Equally important for retention of activity has been the discovery of gelation protocols that avoid the use of exogenous alcohol. Extending established bulk monolith approaches for enzyme encapsulation to thin films involves developing procedures that limit the amount of cracking within the silicate matrix due to rapid evaporation of alcohol (from hydrolysis of semi-metal alkoxide) while maintaining adhesion to a substrate without adding exogenous alcohol.² One thin film strategy involves adding organic functionalized siloxane precursors and forming mixed sols that contain tetramethoxysilane and methyltrimethoxysilane. It is purported that the organic functional groups reduce the degree of cross linking thus relieving some of the internal stress within the pores.³ This approach does limit the addition of exogenous alcohol thus reducing possible denaturation of the enzyme during gelation.

Our initial efforts have focused on encapsulating yeast alcohol dehydrogenase (ADH) with subsequent sensing of short chained alcohols and aldehydes based on fluorescence of the cofactor (NADH).⁴ We investigated ADH/NAD⁺ and ADH/NADH sensing schemes within bulk monoliths and thin films by monitoring the change in fluorescence upon exposure to substrate, as shown in Scheme 1.

Scheme 1



Exposure of alcohol to an ADH/NAD⁺ monolith will generate increases in fluorescence while aldehyde exposure to an ADH/NADH system should cause diminution in fluorescence. Using these changes in fluorescence, we demonstrate sensing of alcohols and aldehydes in aqueous, nonaqueous, and gas phase environments.

Experimental

Reagents: Tetramethoxysilane (TMOS) and methyltrimethoxysilane (m-TMOS) were purchased from Aldrich. Yeast alcohol dehydrogenase (ADH) EC 1.1.1.1 with activity 430 U/mg, nicotinamide adenine dinucleotide (NAD⁺), and reduced nicotinamide adenine dinucleotide (NADH) were obtained from Sigma Chemical Company. All chemicals were used as received and all water used was purified by using a Millipore purification system.

Enzyme Encapsulation: ADH with NADH. A typical encapsulation procedure involved sonication of TMOS (5.12 g, 0.0336 moles) with water (1.20 ml, 0.066 moles) and three drops of HCl solution (0.05 M) at 0°C for 20 minutes to generate a sol.^{1c} Phosphate buffer (1.0 ml, 0.01 M, pH = 8.2) was added to the sol (1.0 ml) which was then shaken vigorously. The enzyme solution (0.20 ml) was then added to the buffered sol and shaken gently. The enzyme solution

usually was comprised of ADH (15 mg/ml) and NADH (7.0×10^{-2} M) in water. After 24 hours of gelling, the parafilm was removed and the monoliths were further aged for a five day period. Phosphate buffer (0.25 ml, 0.1 M, pH = 8.2) was added to each monolith at least 24 hours prior to a fluorescence measurement. The monoliths were further washed with buffer and dried with a tissue before being placed into a fresh cuvette and used for an experiment. Thin films were fabricated by sonicating TMOS (2.00 g, 0.0131 moles), m-TMOS (0.56 g, 0.0041 moles), water (0.60 ml, 0.033 moles) and three drops of HCl solution (0.05 M) for thirty minutes to generate a clear sol. Analogous to the monolith studies, buffer and enzyme solution were added and the resulting solution was spin coated onto precleaned glass slides. The glass slides were stored at 4° C and rinsed with buffer prior to use.

Fluorescence Measurements. Monolith fluorescence measurements were made with a Perkin Elmer MPF 44A spectrofluorimeter that had been computerized by using LabView software. Emission at 450 nm was collected at 90° to excitation at 350 nm. Static fluorescence was collected over time and stored in an ASCII format. A new monolith was used for each substrate sample. The relative velocity of the enzymatic reaction at each aldehyde concentration was determined by a linear fit to the initial fluorescence decrease or to the decrease that followed an induction period. A similar protocol was used with alcohol substrates except that measurements were made at ambient temperature (ca. 23°C) and fluorescence increases were monitored. Thin film fluorescence measurements were made with an ISA Fluorolog 3 instrument operating in a front face geometry. Silicate coated glass slides were placed in cuvettes and static fluorescence was collected over time with correction for excitation lamp drift.

Results and Discussion

Alcohol Sensing: Successful encapsulation of the ADH/NAD⁺ pair within a silica sol-gel polymer was not a foregone conclusion. The hydrolysis process generates four methanol molecules per silicon and it is conceivable that these molecules could react with the dehydrogenase enzyme to generate the fluorescent form of the cofactor. The background signal was suppressed by initially gelling the ADH/NAD⁺ system at a pH where the enzymatic reaction was not favorable followed by post gelation washing with phosphate buffer at pH = 8.8. Monoliths prepared in this fashion exhibited readily measurable increases in fluorescence upon exposure to aqueous solutions of alcohol. As shown in Figure 1, the rate of fluorescence change was correlated to the concentration of substrate in solution. The ability of the silicate polymer to stabilize the enzyme in fairly

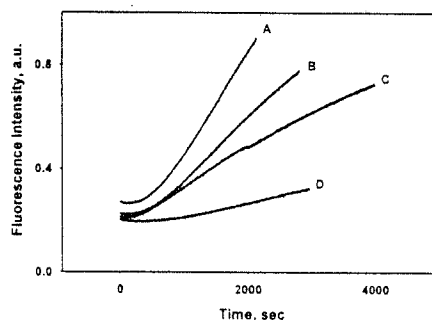


Figure 1: Fluorescence temporal response from ADH and NAD⁺ containing monoliths following liquid phase exposure to: A) 1.0 M, B) 0.50 M, C) 0.20 M, and D) 0.10 M ethanol solutions in phosphate buffer.

innocuous environments such as aqueous buffered solutions encouraged us to examine the enzyme/coenzyme response in more hostile environments such as vapor phase environments or completely nonpolar liquid environments. Exposure of the ADH/NAD⁺ containing monolith to ethanol in liquid hexane yielded a time dependent increase in the NADH fluorescence analogous to the aqueous response. In

contrast, exposure of the free enzyme to hexane solution results in complete denaturation. From additional experiments (encapsulation of a solvatochromic dye within the matrix), it is believed that the siloxane polymer acts as a hydrophobic barrier to the denaturing solvent but allows access of the substrate to the encapsulated enzyme. Exposing the monolith to alcohol vapor also elicited a fluorescence response. The gas phase experiments involved placing monoliths on a raised platform and placing liquid in the bottom of the cuvette. In this fashion, gasohol (commercial gas that contains 10% ethanol) and pure gasoline (commercial gas without added alcohol) were examined. As shown in Figure 2, a fluorescence increase occurs with exposure to gasohol, but no increase accompanies exposure to pure gasoline.

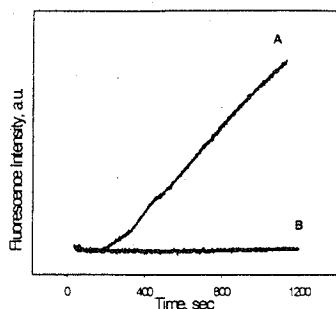


Figure 2. Evolution of fluorescence following gas phase exposure to: A) 10% ethanol in gasoline (gasohol), and B) an alcohol-free sample of gasoline.

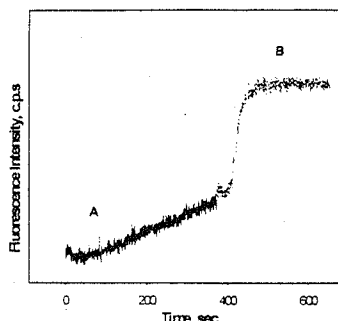


Figure 3. Evolution of fluorescence from a thin film following exposure to: A) residual ethanol vapor, and B) vapor from neat ethanol.

In order to improve upon the time response of the bulk monoliths, thin film versions of the encapsulated ADH/NAD⁺ assembly were fabricated and coated onto glass slide platforms. Preliminary experiments involving thin film exposure to: a) residual ethanol vapor remaining in a cuvette, followed by b) vapor from neat ethanol solution, yielded the gated fluorescence response shown in Figure 3. These preliminary studies suggest that the thin film assemblies have faster time responses than the monolithic assemblies.

Aldehyde Sensing: Successful encapsulation of ADH/NADH for aldehyde sensing is more straightforward than for the alcohol case because aldehyde is not generated during the sol-gel process. Enzyme containing monoliths were fabricated in a similar fashion to that described for the ADH/NAD⁺ monolith system and exposed to substrate in phosphate buffer. Upon exposure to aldehyde, there was a decrease in fluorescence due to the conversion of the fluorescent cofactor NADH to the spectroscopically silent NAD⁺. A more concentrated aldehyde solutions yielded a larger decrease in fluorescence than a less concentrated solution, at least over the short term. Eventually, fluorescence signals from monoliths exposed to the aldehyde of lower concentration diminished to the same level as observed for those exposed to the more concentrated solution. A plot of the rate of fluorescence diminution (initial rate) against the

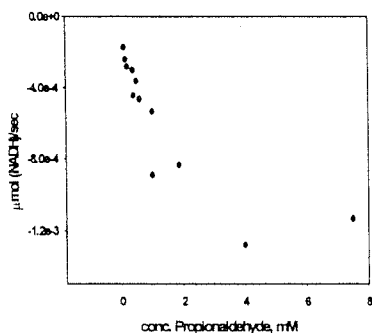


Figure 4. Correlation between initial rate of fluorescence decrease and concentrations of propionaldehyde solutions.

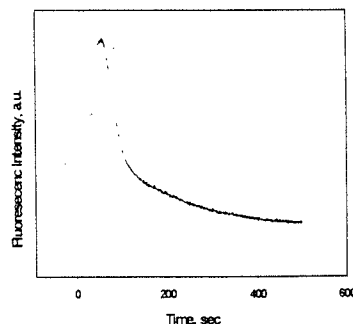


Figure 5. Evolution of fluorescence from a thin film following exposure to propionaldehyde vapor.

propionaldehyde concentration yielded a clear correlation between velocity or rate and substrate concentration, indicating that the assembly indeed can function as a semiquantitative probe of condensed phase aldehyde concentration (Figure 4).

Preliminary thin film studies of a silicate polymer stabilized ADH/NADH system yielded rapid decreases in fluorescence intensity following exposure to the atmosphere above a neat propionaldehyde solution (Figure 5). Ongoing studies involve quantifying the probe response to varying substrate concentrations.

Conclusions

Yeast alcohol dehydrogenase and NADH or NAD⁺ cofactors can be encapsulated in transparent silica monoliths and thin films via sol-gel techniques. Importantly, the techniques are sufficiently mild to permit significant retention of enzymatic activity. By following NADH fluorescence intensity changes, it is possible to use the encapsulated assemblies as both selective chemical probes and sensors. Sol-gel encapsulation additionally provides sufficient stabilization to permit the enzyme and cofactor to be used as vapor-phase alcohol and aldehyde concentration probes. Finally, the encapsulation methodology permits the enzymatic-catalysis/fluorescence-probe technique to be utilized in harsh sample environments, such as liquid hexane, which would otherwise denature the dehydrogenase and render the probe inoperable.

Acknowledgments

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